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Received for publication: 16.8.2013; Accepted in revised form: 13.12.2013

FULL REVIEW

Nephrol Dial Transplant (2014) 29: 1480–1486
doi: 10.1093/ndt/gft521
Advance Access publication 23 January 2014

Defective metabolism in polycystic kidney disease: potential for therapy and open questions

Isaline Rowe and Alessandra Boletta

Division of Genetics and Cell Biology, Dibit San Raffaele Scientific Institute, Milan, Italy

Correspondence and offprint requests to: Alessandra Boletta; E-mail: boletta.alessandra@hsr.it

ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder characterized by bilateral renal cyst formation. The disease is caused by mutations in either the *PKD1* or the *PKD2* gene. Progress has been made in understanding the molecular basis of the disease leading to the general agreement on ADPKD being a loss-of-function disease. Identification of signalling cascades dysfunctional in the cystic epithelia has led to several pre-clinical studies of animal models using a variety of inhibitors to slow disease progression. These were followed by clinical trials, some of which generated promising results, although an approved therapy is still lacking. Here, we summarize and discuss recent work providing evidence that metabolic alterations can be observed in ADPKD. In particular, we will focus our discussion on the potential role of glucose metabolism in the pathogenesis of ADPKD. These recent findings provide a new perspective for the understanding of the pathobiology of ADPKD and open potential new

avenues for therapeutical approaches. At the same time, these studies also raise important and intriguing biological and medical questions that will need to be addressed experimentally prior to embracing a more enthusiastic view of the applicability of the results.

Keywords: ADPKD, glucose metabolism, glycolysis inhibitors, renal cyst, therapeutical approach

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a frequent monogenic disorder, estimated to affect between 1:500 and 1:1000 of the general population [1, 2]. ADPKD accounts alone for 95% of all genetic disorders affecting the kidney and for 8–10% of all individuals in dialysis in the world. The disease is due to mutations in two genes: *PKD1* which is mutated in 85% of cases and *PKD2* in the remaining 15% [1, 2]. The gene products of *PKD1* and *2,* Polycystin-1 and -2, assemble through coiled-coil domains present in their intracellular C-termini to form a functional complex, the activity of which is believed to be essential to prevent renal cystogenesis [3, 4]. Numerous mutations have been identified in ADPKD families along the length of the *PKD1* or *2* genes; most are loss-of-function mutations predicted to inactivate the affected allele [2]. Inherited mutations in *PKD2* lead to a less severe disease phenotype than mutations in the *PKD1* gene and recent studies have uncovered a genotype–phenotype correlation in patients with inherited *PKD1* mutations, whereby truncating mutations lead to a more severe renal phenotype than missense mutations [5].

CYST INITIATION VERSUS CYST EXPANSION: A RELEVANT ISSUE WHEN THINKING OF THERAPIES

Among all cystic kidney diseases, ADPKD is very peculiar because of its dominant inheritance. ADPKD individuals inherit a dominant mutant allele of either the *PKD1* or the *PKD2* gene [1, 2]. ADPKD cysts are focal outpouches originating from any segment of the renal tubule, eventually growing and detaching [6]. Cysts are clonal in nature (derive from a single cell) and a second event besides the first mutated inherited allele is required for a single cell to start forming a cyst [7]. The second event might be a genetic hit affecting the normally inherited allele (two-hit model [7]), or a yet to be identified non-genetic factor causing the amount or function of the PKD proteins to fall below a certain threshold (threshold model [8]). The two mechanisms are not mutually exclusive. The identification of somatic mutations in the *PKD1* gene in patients with inherited *PKD2* mutations illustrates that second hits can affect the threshold of activity of the polycystin complex [9].

Recent studies have also reported the existence of a recessively inherited disease caused by mild mutations in the *PKD1* gene which do not manifest when in heterozygosity, but cause cyst formation only when inherited in homozygosity [8]. Not surprisingly, renal cysts in these individuals are morphologically more similar to the recessive form of PKD, possibly characterized by tubular dilatations rather than focal cysts [10].

Therefore, one could distinguish two distinct phases in focal cystogenesis in ADPKD: cyst initiation and cyst expansion. When thinking of therapies, this distinction might be relevant. Understanding exactly how cysts are initiated would allow to design a therapeutical approach that could correct the primary defect and prevent cysts to form (Figure 1). Understanding the normal function of the polycystins will be key for this purpose and might allow to outline the molecular details that are directly causing a normal tubular cell to grow into an isolated cyst. A therapy able to tackle cyst formation would be optimal, and intense efforts should be devoted to studying the mechanisms of cyst initiation as well as the normal function of the *PKD1* and *2* genes.

If we consider that a relatively low number of cells gives rise to cysts [6], in principle one could also envisage a therapeutic strategy designed to eliminate specifically the altered epithelia at the very beginning, when a few cells compose a cyst and are not even detectable by standard imaging techniques. This type of approach would necessarily rely on the identification of molecules that are selectively toxic for the cystic epithelia, and not for the normal one.

Finally, a third approach and the only one that has so far been pursued is that of slowing down the expansion of already formed cysts by counteracting pro-proliferative pathways upregulated in the cystic epithelia (Figure 1). Even if some of these pathways are unlikely to be initiating events in cyst formation, targeting these might be an equally valid approach in a disease like ADPKD. In this disorder, thousands of cysts can form during the course of an individual's life. However, it was estimated that only 1–5% of all nephrons are affected [1, 6]. The progressive expansion of cysts compresses, damages and eventually replaces the normal tissue leading to kidney failure [1, 11]. In addition, ADPKD is slowly progressive and this process can take up to decades [1]. Therefore, a compound able to slow down cyst expansion should indeed be valuable to postpone kidney failure, even if it is not targeting the primary events of pathogenesis.

ALTERED PATHWAYS AND POTENTIAL THERAPIES

A number of different signalling pathways were reported to be defective in ADPKD cystic epithelia either derived from patients or from animal models. Among these is cAMP which in PKD cystic epithelia is believed to accumulate and cause activation of the ERKs/MAPK pathway [12, 13]. cAMP is probably also involved in driving fluid secretion. Additional pathways that were found up-regulated are the Wnt/beta-catenin and the transcription factor cMyc, STAT1 and STAT3 [14], the mTOR cascade [15], the Hippo pathway [16], TNFα [17] and Sirtuin1 [18]. While several of these pathways were validated in different animal models, it should be noted that for very few of them it was proven that they are directly

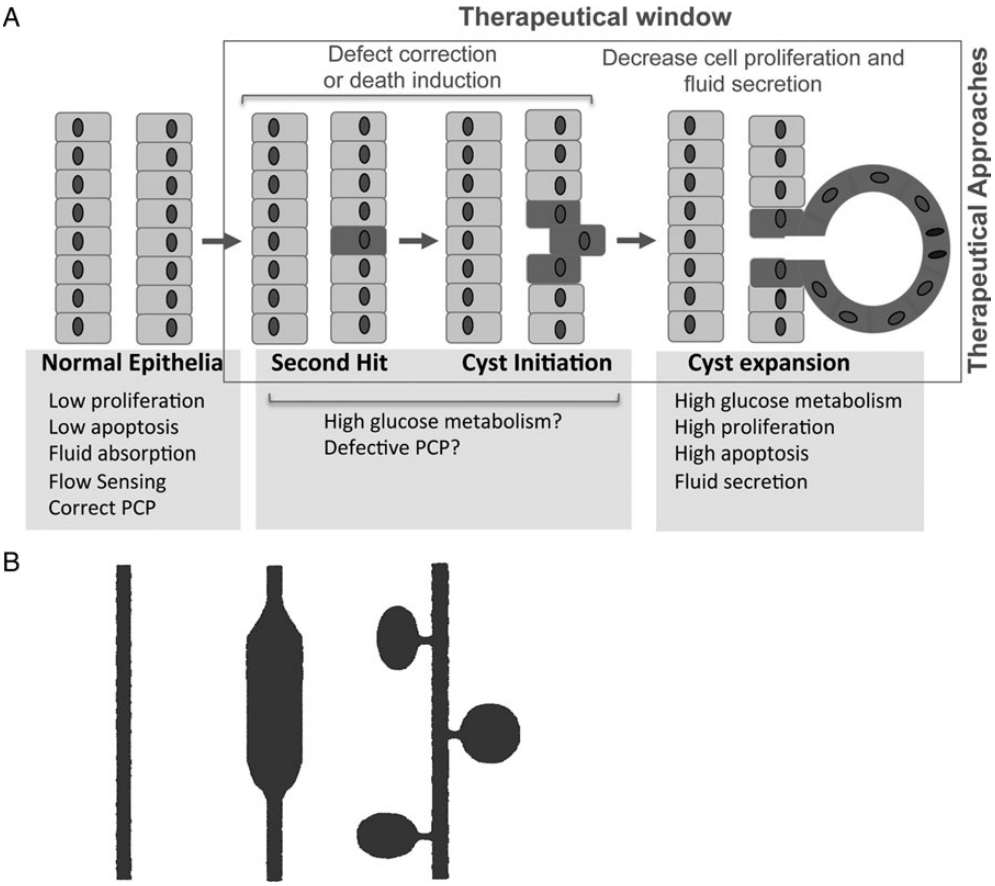

FIGURE 1: Model of focal and clonal expansion of cysts in ADPKD. (**A**) Scheme representing the focal and clonal expansion of a cyst in ADPKD. In normal nephrons (left), epithelial cells are able to sense the flow and to respond by maintaining a normal homeostasis and cellular orientation. After a second event occurs, which could be a somatic second hit (two-hit model) or another event able to lower the *PKD* genes and proteins function below a certain threshold in a single cell (threshold model), the malfunctional cell acquires a number of features many of which are still uncovered (cyst initiation). At this stage, the therapeutical strategy could be to correct the PKD genes altered function or to eliminate specifically the mutated cell using molecules exclusively toxic for the altered cells. After the initial event, mutated cells will expand in a clonal manner forming a cyst (cyst expansion). The epithelial cells lining the cyst present increased proliferation, apoptosis and fluid secretion. During this window of time, the therapeutical strategy consists in slowing down cyst growth targeting cell division or fluid secretion. The defects in metabolic rates of cells could be active in cyst formation or else in cyst expansion and future studies should clarify this. (**B**) Scheme illustrating a normal tubule (left) versus tubular dilatations present in the recessive forms of PKD (middle) and focal cysts characteristics of ADPKD (right).

regulated by the *PKD1* and/or *2* gene and for none of them it has been proved a role in directly causing cyst formation. This, however, did not preclude the testing for inhibition of some of these pathways as a way to slow down cyst expansion as a potential therapeutic approach and several studies have provided encouraging results. In particular, two of these cascades have led to clinical trials in humans. The elevated levels of cAMP in PKD have prompted investigators to use Tolvaptan, an antagonist of the vasopressin receptor that in the collecting ducts is the main driver of cAMP, both in pre-clinical studies and in clinical trials [19, 20]. This leads to promising results, although Tolvaptan is a drug difficult to tolerate for its strong diuretic effects. Furthermore, some liver toxicity might prevent its approval for long-term use in the clinics [20]. The second has been the use of rapamycin and its derivatives both in pre-clinical and in clinical studies [15, 21, 22]. The results of these have been more controversial and less encouraging for reasons possibly linked, at least in part, to its many side effects.

METABOLIC ALTERATIONS IN ADPKD

More recently, two studies have reported that changes in metabolism might be critical in PKD [23, 24]. These results might open new therapeutical opportunities.

Menezes *et al.* [24] have carried out an extensive transcriptomic and urine metabolomic analysis in a murine model of inducible inactivation of *Pkd1* using a Cre recombinase induced at P10, which causes an early-onset polycystic kidney disease in the mouse. The authors have identified metabolic pathways linked with cyst formation and defined HNF4α as a network node [24], in line with the observation of up-regulation of this transcription factor in *Pkd1* null placentas and kidneys [25]. Interestingly, HNF4α has also been described as a regulator of glucose homeostasis, in particular of neoglucogenesis [26] and its activity is among those found altered downstream of the mTOR pathway [27]. Surprisingly, mice

double mutants for both *Pkd1* and HNF4α had a worsened phenotype suggesting that HNF4α might be a modifier of cystogenesis, but also that upregulation of this transcription factor might be part of a protective mechanism. These data illustrate that at least some of the pathways found upregulated in ADPKD might actually be protective and the design of an inhibitory strategy for therapeutic purposes should be carefully tested in pre-clinical studies using adequate animal models.

A second recent study has shown that defective glucose metabolism might be a hallmark of ADPKD which might offer a new opportunity for therapy. Metabolomic profiling using nuclear magnetic resonance (NMR) of the extracellular medium of wild-type and $Pkd1^{-/-}$ cells revealed that the last consume high levels of glucose and preferentially use it in aerobic glycolysis for their energy production. Glucose starvation in these cells resulted in decreased proliferation and increased apoptosis associated with a defect in autophagy. Importantly, NMR analysis of labelled products after ^{13}C-glucose injection in a murine model of ADPKD carrying kidney-specific inactivation of the *Pkd1* gene confirmed increased aerobic glycolysis [23]. Furthermore, key glycolytic genes were found up-regulated in ADPKD cystic epithelia, in cells and in murine cystic kidneys of *Pkd1* mutants (Figure 2) [23]. These data are in line with an up-regulation of mTORC1 in ADPKD, as this complex regulates transcriptional programmes involved in energy metabolism.

Taken together these studies define metabolic regulation as a potential important factor in the pathogenesis and/or progression of polycystic kidney disease and as a potential target for therapy [23, 24].

GLYCOLYSIS AS A TARGET FOR THERAPY?

The principal source of energy of the cell is glucose, which is metabolized through a process named glycolysis (Figure 2). Glucose is transported into cells by facilitative transporters (GLUT1-4) and trapped by a phosphorylation by the enzyme hexokinase (or glucokinase in the liver) [28, 29]. Eight additional enzymatic reactions occur in the cystoplasm leading to generation of two pyruvate molecules [28]. In the presence of oxygen, pyruvate is transported into mitochondria where it is degraded through the tricarboxylic acid (TCA) and generates ~15 molecules of ATP per molecule of pyruvate [28, 29].

In the absence of oxygen, pyruvate is instead converted into lactate in the cytosol. In physiological conditions this process

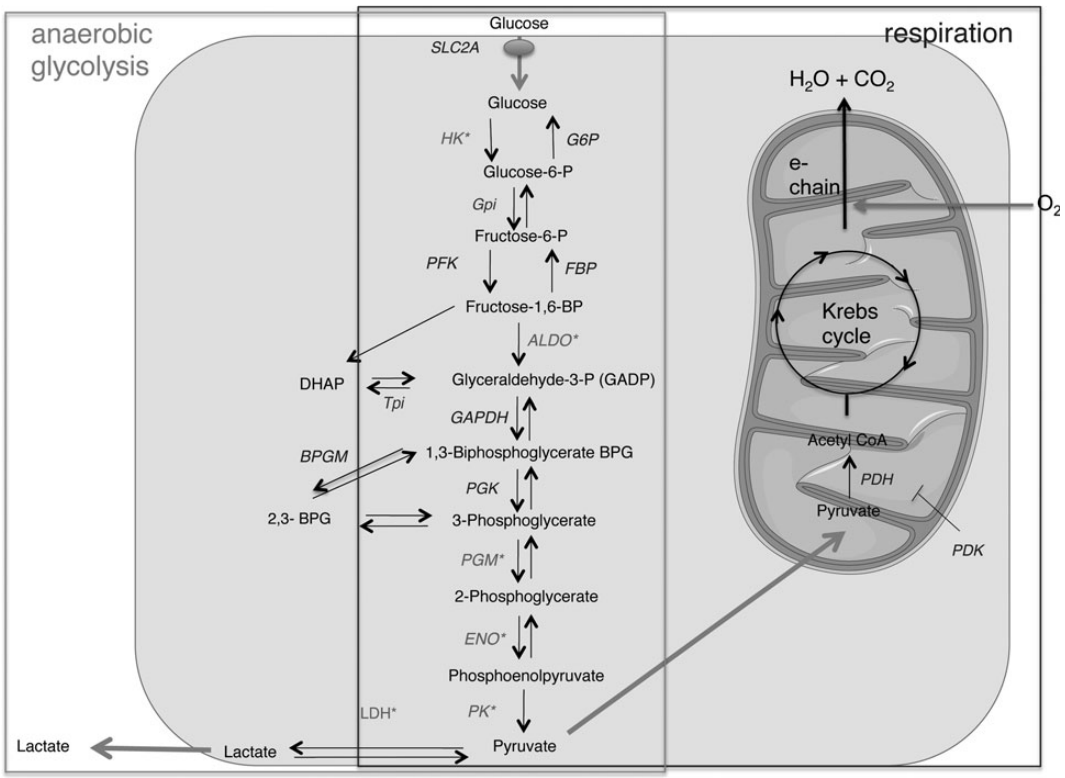

FIGURE 2: Scheme of the glycolytic cascade. Glucose is transported into the cell through transporters (GLUT or SLC2A family) and is transformed by successive enzymatic reactions into pyruvate. In normoxic conditions, pyruvate undergoes a breakdown through respiration in the mitochondria (on the right); it is transported into the mitochondria where it is converted into Acetyl-CoA, which enters the Krebs cycle, also called tricarboxylic acid cycle (TCA cycle) generating energy under the form of ATP through oxidative phosphorylation. In the absence of oxygen anaerobic glycolysis takes place and pyruvate is instead converted into lactate in the cytosol (on the left). In pathological conditions this process is used by cells to generate energy, even when they are in the presence of oxygen, and it is called aerobic glycolysis or Warburg effect. The genes involved in glycolysis differentially expressed in the epithelia lining the cysts of ADPKD kidneys of patients carrying *PKD1* mutations compared with normal kidneys as recently reported [23] are indicated with an asterisk. The image of the mitochondrion was generated using Servier Medical Art, www.servier.com.

is called anaerobic glycolysis (also known as the Pasteur effect) and, although it is not as effective as the TCA cycle in generating ATP, it ensures cell survival in the absence of oxygen [28, 29]. For reasons that are not fully understood, most cancerous cells prefer to use this inefficient process, even when oxygen is available [29]. This pathological condition is called aerobic glycolysis or the Warburg effect and it is one of the hallmarks of cancer [29]. Since generation of energy through aerobic glycolysis is several folds less efficient than using glucose degradative products to fuel the mitochondria, cells typically upregulate the entire process of glucose import and its cytosolic degradation [29]. Many glycolytic enzymes are targets of the hypoxia-inducible factor (HIF1α), which is stabilized in the absence of oxygen. More recently, a network of genes able to regulate glycolysis, but also the pentose phosphate and the lipid biogenesis pathways, was shown to be upregulated downstream of the mTOR complex 1 [27] mostly acting through three transcription factors: SREBP1, HIF1-alpha and HNF4. Finally, other oncogenes also lead to activation of Hif-1 [30] and can lead to activation of glycolysis independently of oxygen levels.

The recent discovery that the Warburg effect is also a feature of PKD opens interesting opportunities for therapy. Several of the enzymes involved in glycolysis were found upregulated in the cystic epithelia derived from ADPKD kidneys (Figure 2) [23]. The study by Rowe *et al.* [23] used 2-deoxy-D-glucose, an analogue of glucose that blocks glycolysis to interfere with the process *in vivo*. This molecule is uptaken by the cells, modified by hexokinase and trapped into the cell, but it cannot be further catabolized in the glycolytic cascade. The use of this compound (at 500 mg/kg) in two orthologous, albeit aggressive, murine models of PKD decreased the kidney/body weight and cysts, providing a strong proof-of-principle that targeting glycolysis might represent a novel therapeutic strategy in ADPKD [23]. Additional studies will be necessary to assess whether doses of this compound comparable with those well tolerated in humans (in a range of 45–63 mg/kg [31, 32]) administered for a long period in slowly progressive PKD mouse models are effective in reducing cyst expansion.

Even if the studies described above provide encouraging results, 2-DG essentially failed in the treatment of cancer, so why should it be more effective in ADPKD? While ADPKD shares some features with cancer, this disease is also profoundly different and the following should be considered: (i) tumours that were selected for treatment with 2DG were very aggressive; (ii) PKD cannot be considered as aggressive as cancer, cyst expansion progresses very slowly and the mild increase in proliferation is not comparable with the hyperproliferation observed in cancer and (iii) the glycolytic switch in PKD cells and kidneys appears to be mild when compared with more aggressive models such as tuberous sclerosis complex mutants (Drusian, Mannella, Musco and Boletta, unpublished) suggesting that the dosages of 2DG that are well tolerated in humans might effectively interfere with the defect [31, 32].

The discovery that the Warburg effect is a feature of PKD also offers the opportunity to test for additional compounds able to inhibit the process at different levels of the glycolytic cascade, many of which are in phase II/III clinical trials (Figure 3) [28]. Furthermore, one of the key enzymes able to sense the energy status of cells, AMPK, was found downregulated in PKD kidneys [23]. In line with this metformin was shown to provide beneficial effects in an experimental model of PKD by acting both on proliferation and secretion of the cystic epithelia [33]. These studies further suggest that multiple molecules can be targeted for inhibition either with individual drugs or by combining them [23]. Thus, in principle, the defective glucose metabolism in PKD might offer several opportunities for intervention (Figure 3) [28].

A recent study described that the nicotinamide adenine dinucleotide-dependent (NAD-dependent) protein deacetylase Sirtuin 1 (SIRT1) is up-regulated in *Pkd1*-mutant cystic kidneys [18]. Of interest, SIRT1 is a metabolic sensor (sensing NAD+) acting on the structure of the chromatin modulating the expression of genes involved in metabolism in different organs [34]. Inactivation of *Sirtuin1* delayed the cystic phenotype in *Pkd1* mutants and treatment with either a pan-sirtuin inhibitor or a specific inhibitor led to delayed renal cyst growth in *Pkd1* mutants [18]. These inhibitors might offer additional opportunities for therapy. It is interesting to note that when glycolysis is high, cells are in need of regenerating the NAD+ molecule in the cytoplasm, and this might in part explain why they convert pyruvate into lactate [35]. It is tempting to speculate that the up-regulated levels of Sirtuin1 in the *Pkd1* mutant animals might reflect a different NAD+/NADH ratio which might be, at least in part, due to the Warburg effect. Further studies might help clarifying if this is the case.

CONCLUSION

In sum, recent studies have reported that metabolic reprogramming might be a feature of ADPKD [23, 24, 36]. While these studies indicate that targeting the metabolic rates of cells or related pathways might offer new therapeutical opportunities, they also raise a number of questions. Are the metabolic alterations observed primary events directly involved in cyst formation, or are they secondary events that manifest only once a cyst has already formed and is expanding? The Warburg effect observed in $Pkd1^{-/-}$ MEFs was not secondary to cell proliferation, since the same metabolic alteration was observed in confluent non-proliferating cells [23]. Furthermore, it is interesting to note that murine models mutants in genes involved in metabolism can manifest with renal cyst formation. For instance, aged transgenic models of HIF2-alpha expressed under the promoter Ksp-Cadherin develop renal cysts, in particular in the cortical region of the kidney [37]. Likewise, mice deficient for *HF*, coding for the Krebs cycle enzyme fumarate hydratase, a tumour suppressor having a key role in energy metabolism, also develop renal cysts when the gene is inactivated specifically in the kidney [38]. These data seem to point to a potential primary role of metabolic alterations in inducing renal cyst formation, although many additional studies will be required to understand if these

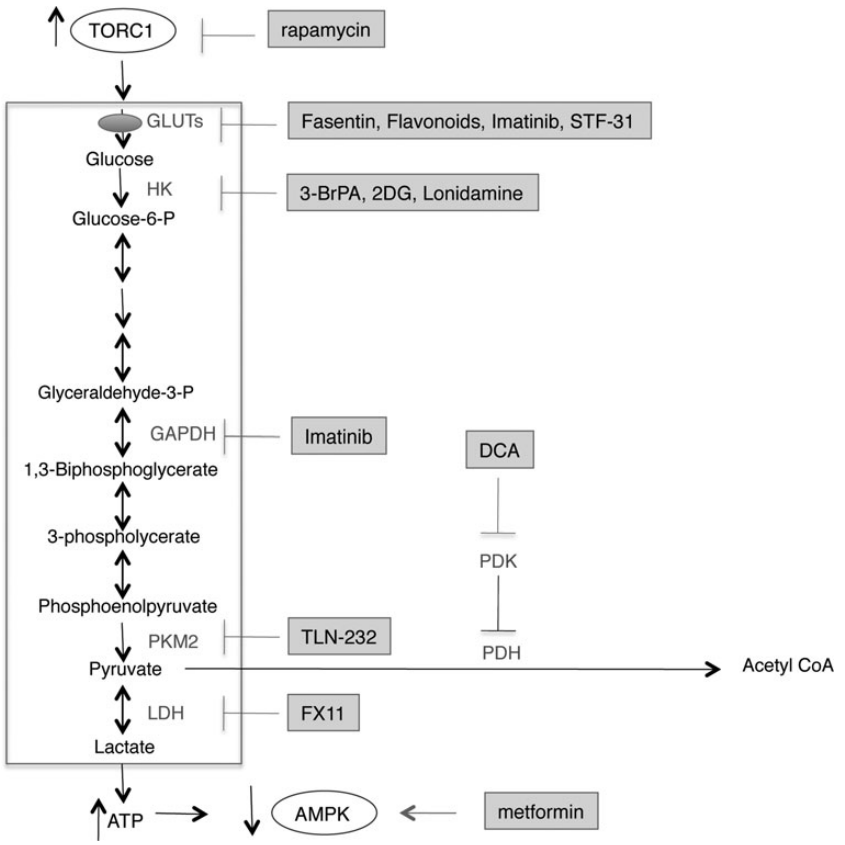

FIGURE 3: Scheme representing glycolysis inhibitors. Glycolysis can be targeted at different levels, compounds are indicated in grey boxes and their corresponding targets are written in grey. During glycolysis, glucose is degraded through successive enzymatic steps generating ATP and this leads to the inactivation of the energy sensor (AMP-Activated Protein Kinase). In order to inhibit this process, the glucose transporters (Glut) and the enzymes HK, GAPDH, PKM2, LDH, PDK have to be inhibited, whereas AMPK has to be activated [28].

metabolic alterations are directly linked to the *PKD1* and *2* genes function and if they can be considered primary events in cystogenesis.

Finally, perhaps the most intriguing question that generates directly from our recent studies is: why ADPKD manifests with cysts and not with tumours? Certainly, cysts can be considered as benign forms of tumours as it has been proposed in a recent commentary, as they share several dysfunctions with tumours including the Warburg effect, considered a hallmark of cancers [36]. However, while for some cystic kidney diseases such as the Von Hipple Lindau the association between cystic lesions and cancer is clear, the incidence of renal cell carcinomas in ADPKD individuals has not been assessed precisely and contradictory results were reported over the years, but it appears that no striking increase over the normal population can be observed [39]. This is very surprising when considering that sporadic cysts are viewed as predisposing events of renal carcinomas [39]. ADPKD cysts, besides being very numerous, are present in the kidneys of affected individuals for decades, they likely have some pro-tumorigenic pathways upregulated, such as the mTORC1 [15] and the Warburg effect [23], and yet the vast majority of them do not transform into cystadenomas and carcinomas. This is, in our view, the most intriguing and challenging observation that deserves a careful attention. Understanding the difference between cystic lesions that transform and those that do not (like in ADPKD) might help better understanding the biology behind both types of diseases and the identification of more specific targets for therapy.

ACKNOWLEDGEMENTS

The authors are grateful to other members of the lab Boletta for helpful discussion, to the Italian Telethon Foundation (GGP12183) and to the associazione italiana rene policistico (AIRP) for funding to A.B.

CONFLICT OF INTEREST STATEMENT

The authors have submitted a patent (US) covering the use of glycolysis inhibitors in ADPKD.

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Received for publication: 17.9.2013; Accepted in revised form: 10.12.2013